

LEUKOTRIENES A₄ AND B₄ STIMULATE THE FORMATION OF CYCLIC AMP IN HUMAN LEUKOCYTES

Hans-Erik CLAEISSON

Department of Physiological Chemistry, Karolinska Institutet, S-104 01 Stockholm, Sweden

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1. Introduction

Recent studies of arachidonic acid metabolism in polymorphonuclear leukocytes (PMNL) have led to the discovery of leukotrienes [1] (fig.1). These compounds have a wide spectrum of biological effects. The activity of most preparations of slow reacting substance of anaphylaxis (SRS-A) is due to leukotriene (LT) C₄ and its metabolites LTD₄ and LTE₄ [1]. LTB₄ induces release of lysosomal enzymes [2,3] and is a potent chemotactic compound [4,5]. The signal for activation of these processes is unknown.

Inflammatory active agents such as latex particles [6], *N*-formyl-methionyl-leucyl-phenylalanine [7,8], C5a [7], calcium ionophore A23187 [8], immune complex [8], zymosan-treated serum [8] and opsinized zymosan [9] have been shown to cause a small rise in cyclic AMP levels, indicating a relationship between neutrophil activation and this cyclic nucleotide. Therefore, we investigated the effects of various leukotrienes on cyclic AMP formation in human PMNL.

This report shows that both LTB₄ and its precursor LTA₄ cause a small rise in cyclic AMP levels which is greatly amplified by pretreatment with RO 20-1724 (an inhibitor of cyclic 3':5'-nucleotide phosphodiesterase).

2. Materials and methods

RO 20-1724 was obtained from Hoffman La

Abbreviations: 5S-HPETE, (5S)-hydroperoxy-eicosatetraenoic acid; LTA₄, 5,6-epoxy-7,9,11,14-eicosatetraenoic acid; LTB₄, (5S), (12R)-dihydroxy-6,8,10,14-eicosatetraenoic acid; LTC₄, (5S)-hydroxy-(6R)-S-glutathionyl-7,9,11,14-eicosatetraenoic acid; RP 20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazol-idinone; cyclic AMP, adenosine 3':5'-monophosphate

Roche (Basel); Dextran T-500 from Pharmacia Fine Chemicals (Uppsala) and Hypaque/Ficoll from Nyegaard and Co. (Oslo).

2.1. Separation of leukocytes

PMNL were either isolated as in [10] or in the following way: Blood from healthy donors was collected from the antecubital vein in tubes containing EDTA (final conc. 6 mM). After centrifugation at 200 × *g* for 15 min, the lower phase was mixed with 2% Dextran T-500 solution containing 0.9% of NaCl. After 30 min, the upper layer was gently removed and centrifuged at 200 × *g* for 15 min at 0°C. Supernatant fluid was aspirated and erythrocytes were removed by hypotonic lysis (0.15 M NH₄Cl, 37°C for 7 min). The cell-rich solution was then fractionated on a Hypaque/Ficoll gradient [11]. After centrifugation at 300 × *g* for 40 min, the pellets were resuspended in phosphate-buffered saline (pH 7.4). Similar results were obtained using either of these preparations of PMNL.

2.2. Leukotrienes preparation

Synthetic LTA₄ was kindly given by Dr E. J. Corey. LTB₄, 20-OH-LTB₄ and 20-COOH-LTB₄ were prepared as in [12,13]. LTC₄ was generated biosynthetically [14].

2.3. Experimental conditions and quantitative analyses of cyclic AMP

Cell suspensions (10–30 × 10⁶ cells/ml) were preincubated for 2 min at 37°C. Compounds to be tested were added in 1–2 μl ethanol to 1 ml cell suspension. Incubations were terminated by adding 1 vol. 10% trichloroacetic acid. Cyclic AMP levels were determined using the protein binding technique in [15]. Prior to analyses the samples were purified [16].

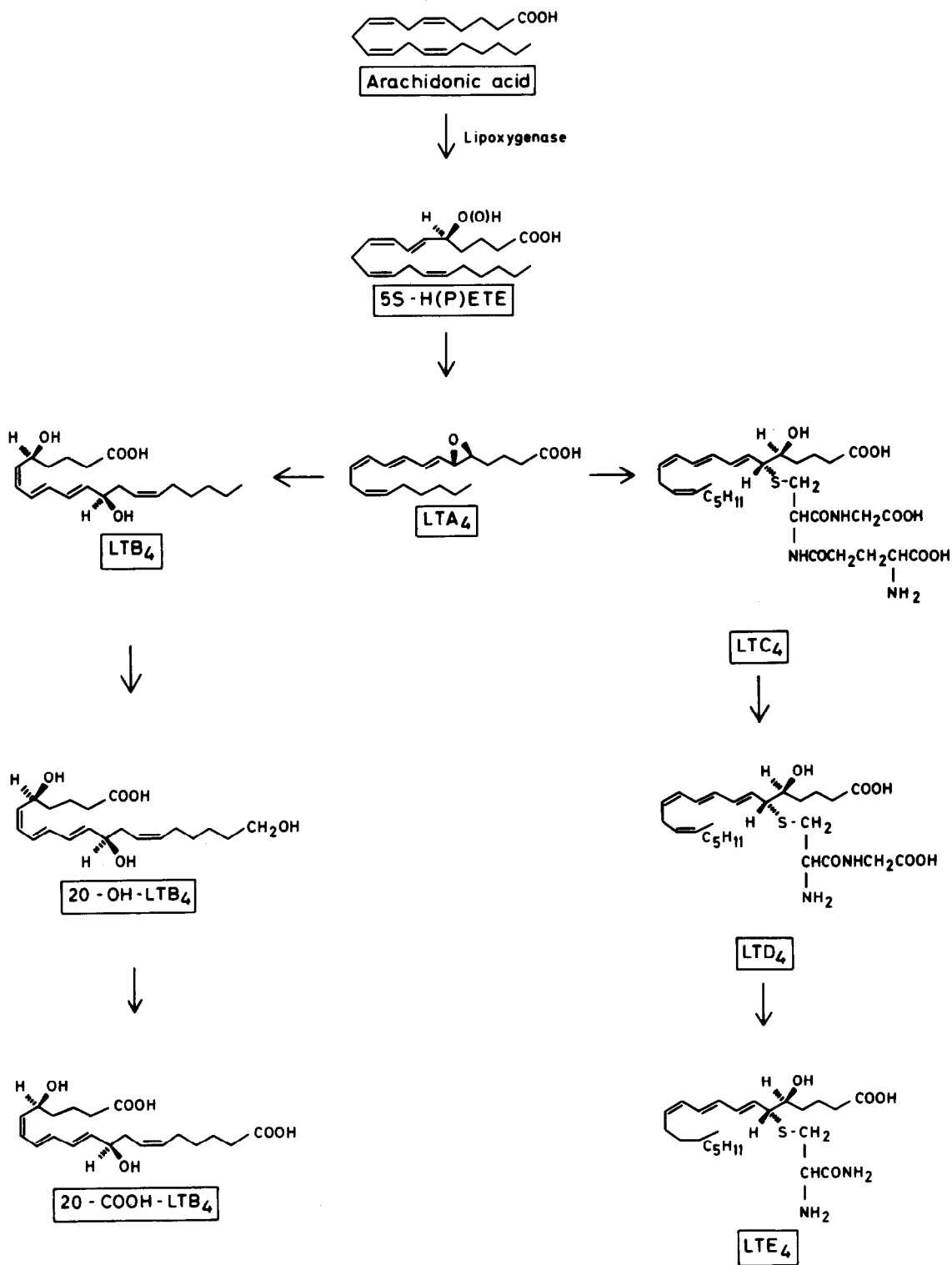


Fig.1. Transformation of arachidonic acid in human PMNL.

3. Results and discussion

A suspension of washed human PMNL was incubated with either LTA₄, LTB₄, or LTC₄. LTA₄ and LTB₄ caused a small rise in cyclic AMP levels (table 1).

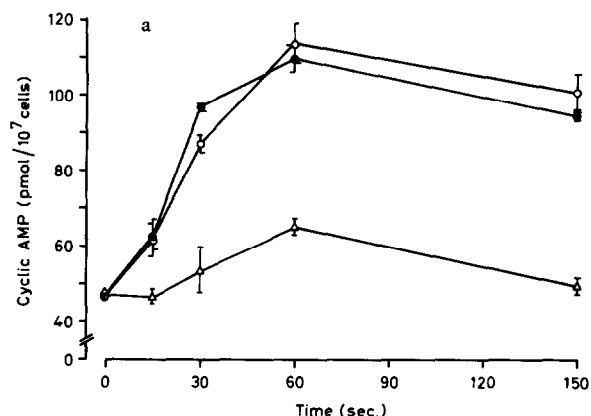
Table 1
Effects of LTA₄, LTB₄ and LTC₄ on the levels of cyclic AMP in human PMNL

Exp.	Addition (ng/ml)	Cyclic AMP (pmol/10 ⁷ cells)
1	None	22.3 ± 2.4
	LTA ₄ (300)	25.6 ^a ± 1.9
	LTB ₄ (100)	28.7 ^b ± 1.8
	LTC ₄ (350)	21.1 ^{n.s.} ± 1.1
2	None	36.0 ± 3.9
	LTA ₄ (100)	71.5 ^c ± 3.1
	LTB ₄ (100)	90.0 ^c ± 1.8
	LTC ₄ (350)	37.0 ^{n.s.} ± 3.2

Exp. 1: Human PMNL were incubated with either LTA₄, LTB₄ or LTC₄ for 30 s prior to cyclic AMP analyses

Exp. 2: Human PMNL were pretreated 2 min with RO 20-1724 (0.1 mM). Thereafter the cells were incubated with either LTA₄, LTB₄ or LTC₄ for 1 min

Each value represents the mean value ± SD from triplicate determination on each of 6 samples (exp. 1) or 3 samples (exp. 2). Statistical differences were performed using Student's t-test: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$; n.s., not significant



These effects were greatly amplified in the presence of RO 20-1724 (an inhibitor of cyclic 3':5'-nucleotide phosphodiesterase, EC 3.1.4.17). Similar results were obtained using another phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (not shown). No effect on cyclic AMP synthesis was observed with LTC₄ (table 1). LTA₄ and LTB₄ rapidly elevated the levels of cyclic AMP in human PMNL (fig.2a). A maximal effect was observed after 1 min. Similar time-courses have been reported in PMNL incubated with *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), ionophore A23187, immune complex or zymosan-treated serum after pretreatment with prostaglandin E₁ [8] or a phosphodiesterase inhibitor [17]. Fig.2b shows that LTB₄ and LTA₄ were about equally potent as stimulators of cyclic AMP formation. The effect of LTB₄ on cyclic AMP synthesis is in agreement with the earlier observations that agents which possess the ability to induce superoxide anion (O₂⁻) production and secretion of azurophil granules also cause a small increment in cyclic AMP levels [8].

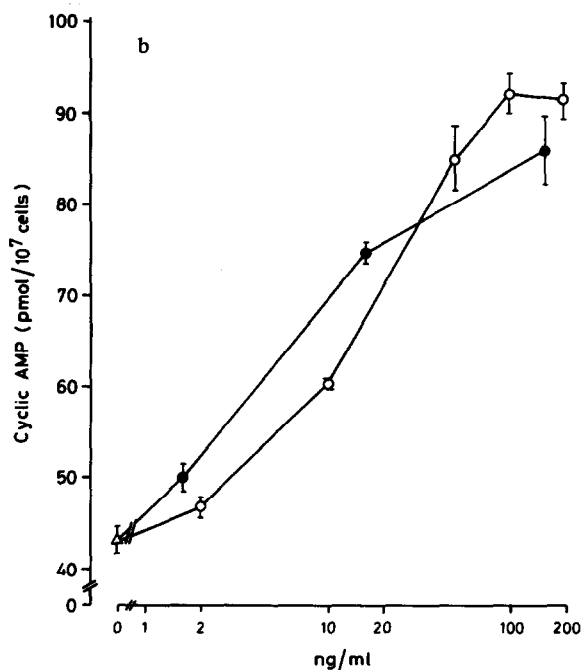


Fig.2. Effects of LTA₄ and LTB₄ on the levels of cyclic AMP in human PMNL: (a) Time-courses of the effects of LTA₄ (100 ng/ml) or LTB₄ (100 ng/ml) on the formation of cyclic AMP in the presence of RO 20-1724 (0.1 mM); (b) Dose-response curves showing the effects of LTA₄ and LTB₄ on cyclic AMP synthesis in the presence of RO 20-1724 (0.1 mM); (Δ) RO 20-1724; (●) LTA₄ + RO 20-1724; (○) LTB₄ + RO 20-1724. The cells were preincubated for 2 min with RO 20-1724 at 37°C. Thereafter the cells were incubated with LTA₄ or LTB₄ and analyzed for cyclic AMP content after indicated time (a) or 1 min (b). Each value represents the mean value ± SD from triplicate determination on each of 2 samples.

However, elevated cyclic AMP levels obtained after stimulation with various prostaglandins or phosphodiesterase inhibitors lead to inhibition in [10] of LTB₄ synthesis and in [8,18] of enzyme release. Thus, an increment of cyclic AMP levels seems to be associated both with stimulation and inhibition of granulocyte function. A possible explanation for this is that agents which activate neutrophils increase the levels of cyclic AMP in certain compartments [9].

LTB₄ has been shown to release prostaglandins from guinea pig lung [19]. Therefore, the effect of indomethacin and aspirin on the stimulatory action of LTB₄ on cyclic AMP formation was investigated. Human PMNL were incubated with indomethacin (1 μ M) or aspirin (22 μ M) in combination with RO 20-1724 (0.1 mM) for 2 min prior to the addition of LTB₄. In the absence of prostaglandin synthesis inhibitors, LTB₄ (100 ng/ml) increased the levels of cyclic AMP from 29.0 ± 0.04 to 56.2 ± 2.9 pmol/ 10^7 cells after 1 min. In the presence of indomethacin or aspirin, LTB₄ increased the cyclic AMP content to 50.7 ± 1.5 and 55.2 ± 3.1 pmol/ 10^7 cells, respectively, indicating that this effect on cyclic AMP was not mediated by prostaglandins.

Omega oxidation of LTB₄ leads to formation of 20-OH-LTB₄ and 20-COOH-LTB₄ [13] (fig.1). These compounds were less effective than LTB₄ in stimulating cyclic AMP formation. In the presence of RO 20-1724 (0.1 mM), LTB₄ (100 ng/ml) elevated the basal level of cyclic AMP, 31.3 ± 2.5 pmol/ 10^7 cells, to 70.3 ± 6.4 pmol/ 10^7 cells whereas 20-OH-LTB₄ (100 ng/ml) and 20-COOH-LTB₄ (100 ng/ml) increased the levels to 51.1 ± 1.7 and 45.5 ± 4.5 pmol/ 10^7 cells, respectively, after 1 min incubation.

This study suggests that cyclic AMP is associated with the biological effects of LTB₄ in human polymorphonuclear leukocytes. We are further investigating the relationship between cyclic AMP levels and degranulation in LTB₄-stimulated leukocytes.

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